FIG. 1

GCTTCCCGAGGCTCCGCACCAGCCGCGCTTCTGTCCGCCTGCAGGGCATTCCA
GAAAGATGAGGATATTTGCTGTCTTTATATTCATGACCTACTGGCATTTGCTG
AACGCATTTACTGTCACGGTTCCCAAGGACCTATATGTGGTAGAGTATGGTA
GCAATATGACAATTGAATGCAAATTCCCAGTAGAAAAACAATTAGACCTGGC
TGCACTAATTGTCTATTGGGAAATGGAGGATAAGAACATTATTCAATTTGTGC
ATGGAGAGGAAGACCTGAAGGTTCAGCATAGTAGCTACAGACAGAGGGCCC
GGCTGTTGAAGGACCAGCTCTCCCTGGGAAATGCTGCACTTCAGATCACAGA
TGTGAAATTGCAGGATGCAGGGGTGTACCGCTGCATGATCAGCTATGGTGGT
GCCGACTACAAGCGAATTACTGTGAAAGTCAATGCCCCATACAACAAAATCA
ACCAAAGAATTTTGGTTGTGGATCCAGTCACCTCTGAACATGAACTGACATGT
CAGGCTGAGGGCTACCCCAAGGCCGAAGTCATCTGGACAAGCAGTGACCATC
AAGTCCTGAGTGGTAAGACCACCACCACCAATTCCAAGAGAGAGGAGAAGC
TTTTCAATGTGACCAGCACACTGAGAATCAACACAACAACTAATGAGATTTT
CTACTGCACTTTTAGGAGATTAGATCCTGAGGAAAACCATACAGCTGAATTG
GTCATCCCAGGTAATATTCTGAATGTGTCCATTAAAATATGTCTAACACTGTC
CCCTAGCACCTAGCATGATGTCTGCCTATCATAGTCATTCAGTGATTGTTGAA
TAAATGAATGAATGAATAACACTATGTTTACAAAATATATCCTAATTCCTCAC
CTCCATTCATCCAAACCATATTGTTACTTAATAAACATTCAGCAGATATTTAT
GGAATAAAAAAAAAAAAAAAAAAAA

FIG. 2


CGAGGCTCCGCACCAGCCGCGCTTCTGTCCGCCTGCAGGGCATTCCAGAAAGA
TGAGGATATTTGCTGTCTTTATATTCATGACCTACTGGCATTTGCTGAACGCATT
TACTGTCACGGTTCCCAAGGACCTATATGTGGTAGAGTATGGTAGCAATATGAC
AATTGAATGCAAATTCCCAGTAGAAAAACAATTAGACCTGGCTGCACTAATTGT
CTATTGGGAAATGGAGGATAAGAACATTATTCAATTTGTGCATGGAGAGGAAG
ACCTGAAGGTTCAGCATAGTAGCTACAGACAGAGGGCCCGGCTGTTGAAGGAC
CAGCTCTCCCTGGGAAATGCTGCACTTCAGATCACAGATGTGAAATTGCAGGAT
GCAGGGGTGTACCGCTGCATGATCAGCTATGGTGGTGCCGACTACAAGCGAAT
TACTGTGAAAGTCAATGCCCCATACAACAAAATCAACCAAAGAATTTTGGTTGT
GGATCCAGTCACCTCTGAACATGAACTGACATGTCAGGCTGAGGGCTACCCCA
AGGCCGAAGTCATCTGGACAAGCAGTGACCATCAAGTCCTGAGTGGTAAGACC
ACCACCACCAATTCCAAGAGAGAGGAGAAGCTTTTCAATGTGACCAGCACACT
GAGAATCAACACAACAACTAATGAGATTTTCTACTGCACTTTTAGGAGATTAGA
TCCTGAGGAAAACCATACAGCTGAATTGGTCATCCCAGAACTACCTCTGGCACA
TCCTCCAAATGAAAGGACTCACTTGGTAATTCTGGGAGCCATCTTATTATGCCTT
GGTGTAGCACTGACATTCATCTTCCGTTTAAGAAAAGGGAGAATGATGGATGT
GAAAAAATGTGGCATCCAAGATACAAACTCAAAGAAGCAAAGTGATACACATTT
GGAGGAGACGTAATCCAGCATTGGAACTTCTGATCTTCAAGCAGGGATTCTCA
ACCTGTGGTTTAGGGGTTCATCGGGGCTGAGCGTGACAAGAGGAAGGAATGG
GCCCGTGGGATGCAGGCAATGTGGGACTTAAAAGGCCCAAGCACTGAAAATG
GAACCTGGCGAAAGCAGAGGAGGAGAATGAAGAAAGATGGAGTCAAACAGGG
AGCCTGGAGGGAGACCTTGATACTTTCAAATGCCTGAGGGGCTCATCGACGCC
TGTGACAGGGAGAAAGGATACTTCTGAACAAGGAGCCTCCAAGCAAATCATCC
ATTGCTCATCCTAGGAAGACGGGTTGAGAATCCCTAATTTGAGGGTCAGTTCCT
GCAGAAGTGCCCTTTGCCTCCACTCAATGCCTCAATTTGTTTTCTGCATGACTGA
GAGTCTCAGTGTTGGAACGGGACAGTATTTATGTATGAGTTTTTCCTATTTATTT
TGAGTCTGTGAGGTCTTCTTGTCATGTGAGTGTGGTTGTGAATGATTTCTTTTGA
AGATATATTGTAGTAGATGTTACAATTTTGTCGCCAAACTAAACTTGCTGCTTAA
TGATTTGCTCACATCTAGTAAAACATGGAGTATTTGTAAAAAAAAAAAAAAA

FIG. 3

292 secreted (245 amino acids)

Signal/IgV/IgC/hydrophilic tail
(a) (b) (c) (d)
Ig cysteines in large bold

MRIFAVFIFMTYWHLLNA (signal)

FTVTVPKDLYVVEYGSNMTIE**C**KFPVEKQLDLAALIVYWEMEDKN
IIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQD
AGVYR**C**MISYGGADYKRITVKVNAPY (IgV)

NKINQRILVVDPVTSEHELT**C**QAEGYPKAEVIWTSSDHQVLSGKT
TTTNSKREEKLFNVTSTLRINTTTNEIFY**C**TFRRLDPEENHTAEL
VIP (IgC)

GNILNVSIKICLTLSPST (hydrophilic tail)

FIG. 4

292 membrane (290 amino acids)

Signal/IgV/IgC/transmembrane (underlined) plus cytoplasmic

Ig cysteines in large bold

MRIFAVFIFMTYWHLLNA (signal)

FTVTVPKDLYVVEYGSNMTIE**C**KFPVEKQLDLAALIVYWEMEDKN
IIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQD
AGVYR**C**MISYGGADYKRITVKVNAPY (IgV)

NKINQRILVVDPVTSEHELT**C**QAEGYPKAEVIWTSSDHQVLSGKT
TTTNSKREEKLFNVTSTLRINTTTNEIFY**C**TFRRLDPEENHTAEL
VIP (IgC)

ELPLAHPPNER<u>THLVILGAILLCLGVALTFIF</u>RLRKGRMMDVKKC
GIQDTNSKKQSDTHLEET (transmembrane plus cytoplasmic)

FIG. 5A


AGATAGTTCCCAAAACATGAGGATATTTGCTGGCATTATATTCACAGCCTGC
TGTCACTTGCTACGGGCGTTTACTATCACGGCTCCAAAGGACTTGTACGTG
GTGGAGTATGGCAGCAACGTCACGATGGAGTGCAGATTCCCTGTAGAACG
GGAGCTGGACCTGCTTGCGTTAGTGGTGTACTGGGAAAAGGAAGATGAGC
AAGTGATTCAGTTTGTGGCAGGAGAGGAGGACCTTAAGCCTCAGCACAGCA
ACTTCAGGGGGAGAGCCTCGCTGCCAAAGGACCAGCTTTTGAAGGGAAAT
GCTGCCCTTCAGATCACAGACGTCAAGCTGCAGGACGCAGGCGTTTACTGC
TGCATAATCAGCTACGGTGGTGCGGACTACAAGCGAATCACGCTGAAAGTC
AATGCCCCATACCGCAAAATCAACCAGAGAATTTCCGTGGATCCAGCCACTT
CTGAGCATGAACTAATATGTCAGGCCGAGGGTTATCCAGAAGCTGAGGTAA
TCTGGACAAACAGTGACCACCAACCCGTGAGTGGGAAGAGAAGTGTCACCA
CTTCCCGGACAGAGGGGATGCTTCTCAATGTGACCAGCAGTCTGAGGGTCA
ACGCCACAGCGAATGATGTTTTCTACTGTACGTTTTGGAGATCACAGCCAG
GGCAAAACCACACAGCGGAGCTGATCATCCCAGAACTGCCTGCAACACATC
CTCCACAGAACAGGACTCACTGGGTGCTTCTGGGATCCATCCTGTTGTTCC
TCATTGTAGTGTCCACGGTCCTCCTCTTCTTGAGAAAACAAGTGAGAATGCT
AGATGTGGAGAAATGTGGCGTTGAAGATACAAGCTCAAAAAACCGAAATGA
TACACAATTCGAGGAGACGTAAGCAGTGTTGAACCCTCTGATCGTCGATTG
GCAGCTTGTGGTCTGTGAAAGAAAGGGCCCATGGGACATGAGTCCAAAGAC
TCAAGATGGAACCTGAGGGAGAGAACCAAGAAAGTGTTGGGAGAGGAGCC
TGGAACAACGGACATTTTTTCCAGGGAGACACTGCTAAGCAAGTTGCCCAT
CAGTCGTCTTGGGAAATGGATTGAGGGTTCCTGGCTTAGCAGCTGGTCCTT
GCACAGTGACCTTTTCCTCTGCTCAGTGCCGGGATGAGAGATGGAGTCATG
AGTGTTGAAGAATAAGTGCCTTCTATTTATTTTGAGTCTGTGTGTTCTCACTT
TGGGCATGTAATTATGACTGGTGAATTCTGACGACATGATAGATCTTAAGAT
GTAGTCACCAAACTCAACTGCTGCTTAGCATCCTCCGTAACTACTGATACAA
GCAGGGAACACAGAGGTCACCTGCTTGGTTTGACAGGCTCTTGCTGTCTGA
CTCAAATAATCTTTATTTTTCAGTCCTCAAGGCTCTTCGATAGCAGTTGTTCT
GTATCAGCCTTATAGGTGTCAGGTATAGCACTCAACATCTCATCTCATTACA
ATAGCAACCCTCATCACCATAGCAACAGCTAACCTCTGTTATCCTCACTTCA
TAGCCAGGAAGCTGAGCGACTAAGTCACTTGCCCACAGAGTATCAGCTCTC
AGATTTCTGTTCTTCAGCCACTGTCCTTTCAGGATAGAATTTGTCGTTAAGAA
ATTAATTTAAAAACTGATTATTGAGTAGCATTGTATATCAATCACAACATGCC
TTGTGCACTGTGCTGGCCTCTGAGCATAAAGATGTACGCCGGAGTACCGGT
CGGACATGTTTATGTGTGTTAAATACTCAGAGAAATGTTCATTAACAAGGAG
CTTGCATTTTAGAGACACTGGAAAGTAACTCCAGTTCATTGTCTAGCATTAC
ATTTACCTCATTTGCTATCCTTGCCATACAGTCTCTTGTTCTCCATGAAGTGT
CATGAATCTTGTTGAATAGTTCTTTTATTTTTTAAATGTTTCTATTTAAATGATA
TTGACATCTGAGGCGATAGCTCAGTTGGTAAAACCCTTTCCTCACAAGTGTG
AAACCCTGAGTCTTATCCCTAGAACCCACATAAAAAACAGTTGCGTATGTTT
GTGCATGCTTTTGATCCCAGCACTAGGGAGGCAGAGGCAGGCAGATCCTG
AGCTCTCATTGACCACCCAGCCTAGCCTACATGGTTAGCTCCAGGCCTACA
GGAGCTGGCAGAGCCTGAAAAACGATGCCTAGACACACACACACACACACA
CACACACACACACACACACACACACACCATGTACTCATAGACCTAAGTGCACC
CTCCTACACATGCACACACATACAATTCAAACACAAATCAACAGGGAATTGT

FIG. 5B

CTCAGAATGGTCCCCAAGACAAAGAAGAAGAAAAACACCAAACCAGCTCTA
TTCCCTCAGCCTATCCTCTCTACTCCTTCCTAGAAGCAACTACTATTGTTTTT
GTATATAAATTTACCCAACGACAGTTAATATGTAGAATATATATTAAAGTGTC
TGTCAATATATATTATCTCTTTCTTTCTTTCTTCCTTTCTTTCTTTCTTTCTTTC
TTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTCCTTCCTTCCTTCCTTCCTTC
CTTCCTTCCTTCCTTTCTTTCTTTCTTTCTTTTTTTCTGTCTATCTGTACCTAAA
TGGTTGCTCACTATGCATTTTCTGTGCTCTTCGCCCTTTTTATTTAATGTATG
GATATTTATGCTGCTTCCAGAATGGATCTAAAGCTCTTTGTTTCTAGGTTTTC
TCCCCCATCCTTCTAGGCATCTCTCACACTGTCTAGGCCAGACACCATGTCT
GCTGCCTGAATCTGTAGACACCATTTATAAAGCACGTACTCACCGAGTTTGT
ATTTGGCTTGTTCTGTGTCTGATTAAAGGGAGACCATGAGTCCCCAGGGTA
CACTGAGTTACCCCAGTACCAAGGGGGAGCCTTGTTTGTGTCTCCATGGCA
GAAGCAGGCCTGGAGCCATTTTGGTTTCTTCCTTGACTTCTCTCAAACACAG
ACGCCTCACTTGCTCATTACAGGTTCTCCTTTGGGAATGTCAGCATTGCTCC
TTGACTGCTGGCTGCCCTGGAAGGAGCCCATTAGCTCTGTGTGAGCCCTTG
ACAGCTACTGCCTCTCCTTACCACAGGGGCCTCTAAGATACTGTTACCTAGA
GGTCTTGAGGATCTGTGTTCTCTGGGGGGAGGAAAGGAGGAGGAACCCAG
AACTTTCTTACAGTTTTCCTTGTTCTGTCACATGTCAAGACTGAAGGAACAG
GCTGGGCTACGTAGTGAGATCCTGTCTCAAAGGAAAGACGAGCATAGCCGA
ACCCCCGGTGGAACCCCCTCTGTTACCTGTTCACACAAGCTTATTGATGAGT
CTCATGTTAATGTCTTGTTTGTATGAAGTTTAAGAAAATATCGGGTTGGGCAA
CACATTCTATTTATTCATTTTATTTGAAATCTTAATGCCATCTCATGGTGTTGG
ATTGGTGTGGCACTTTATTCTTTTGTGTTGTGTATAACCATAAATTTTATTTTG
CATCAGATTGTCAATGTATTGCATTAATTTAATAAATATTTTTATTTATTAAAAA
AAAAAAAAAAAAAAA

FIG. 6

MRIFAGIIFTACCHLLRAFTITAPKDLYVVEYGSNVTMECRFPVERELDLLALVVYWEKEDEQVIQFVAGEE
DLKPQHSNFRGRASLPKDQLLKGNAALQITDVKLQDAGVYCCIISYGGADYKRITLKVNAPYRKINQRISV
DPATSEHELICQAEGYPEAEVIWTNSDHQPVSGKRSVTTSRTEGMLLNVTSSLRVNATANDVFYCTFWR
SQPGQNHTAELIIPELPATHPPQNRTHWVLLGSILLFLIVVSTVLLFLRKQVRMLDVEKCGVEDTSSKNRN
DTQFEET.

FIG. 7

mB7-4 vs. hB7-4

69% identity

```
mB7-4    1   MRIFAGIIFTACCHLLRAFTITAPKDLYVVEYGSNVTMECRFPVERELDLLALVVYWEKE 60
             MRIFA  IF    HLL AFT+T PKDLYVVEYGSN+T+EC+FPVE++LDL AL+VYWE E
hB7-4    1   MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEME 60

mB7-4   61   DEQVIQFVAGEEDLKPQHSNFRGRASLPKDQLLKGNAALQITDVKLQDAGVYCCIISYGG 120
             D+ +IQFV GEEDLK QHS++R RA L KDQL  GNAALQITDVKLQDAGVY C+ISYGG
hB7-4   61   DKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGG 120

mB7-4  121   ADYKRITLKVNAPYRKINQRI-SVDPATSEHELICQAEGYPEAEVIWTNSDHQPVSGKRS 179
             ADYKRIT+KVNAPY KINQRI  VDP TSEHEL CQAEGYP+AEVIWT+SDHQ +SGK +
hB7-4   121   ADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTT 180

mB7-4   180   VTTSRTEGMLLNVTSSLRVNATANDVFYCTFWRSQPGQNHTAELIIPELPATHPPQNRTH 239
              T S+ E  L NVTS+LR+N T N++FYCTF R  P +NHTAEL+IPELP  HPP  RTH
hB7-4   181   TTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTH 240

mB7-4   240   WVLLGSILLFLIVVSTVLLFLRKQVRMLDVEKCGVEDTSSKNRNDTQFEET 290
              V+LG+ILL L V  T +  LRK  RM+DV+KCG++DT+SK ++DT  EET
hB7-4   241   LVILGAILLCLGVALTFIFRLRKG-RMMDVKKCGIQDTNSKKQSDTHLEET 290
```

FIG. 8

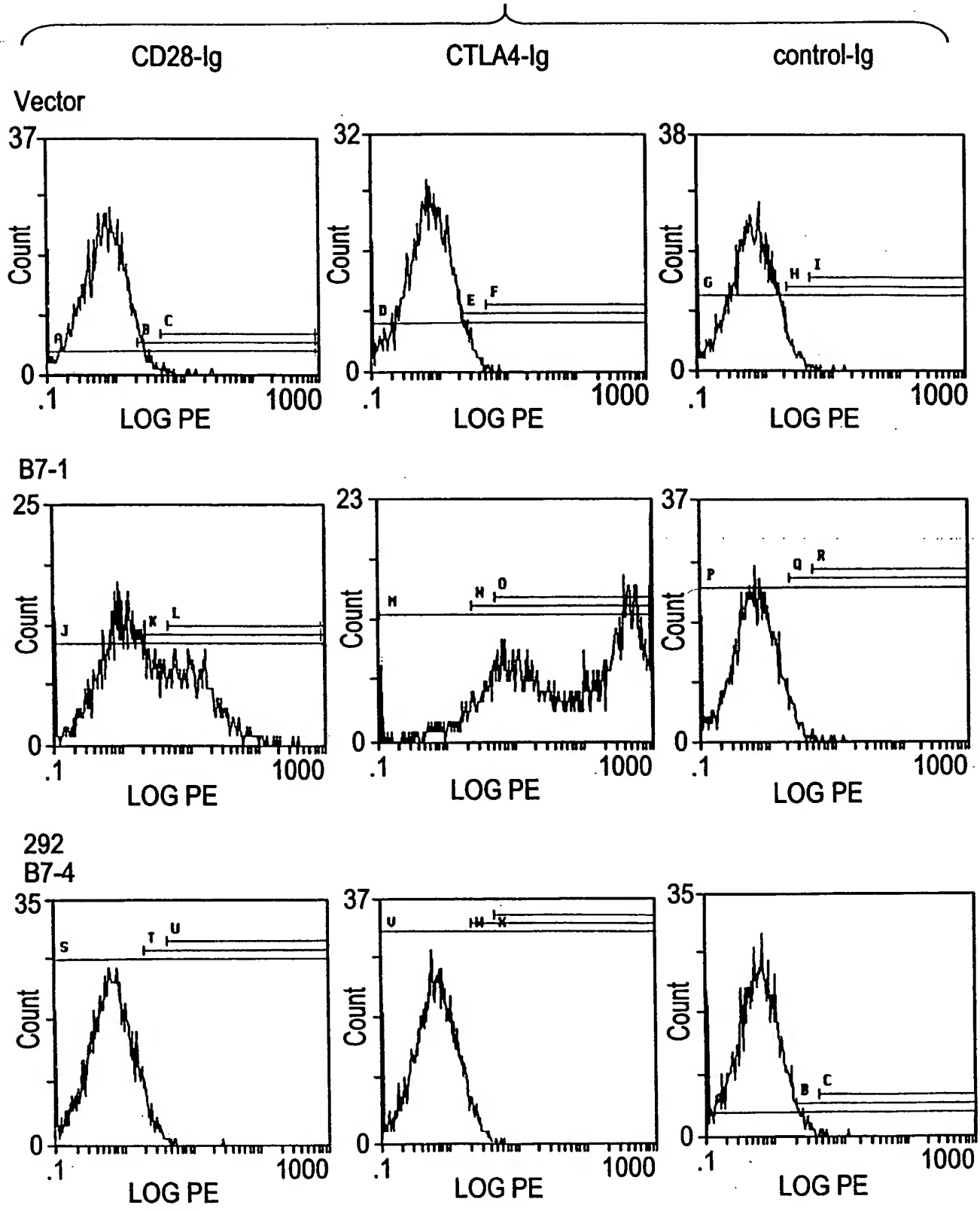

CD28-Ig
CTLA4-Ig
control-Ig
Vector
37
32
38
Count
0
.1
1000
LOG PE
A
B
C
D
E
F
G
H
I
B7-1
25
23
37
J
K
L
M
N
O
P
Q
R
292
B7-4
35
37
35
S
T
U
V
W
X

FIG. 9

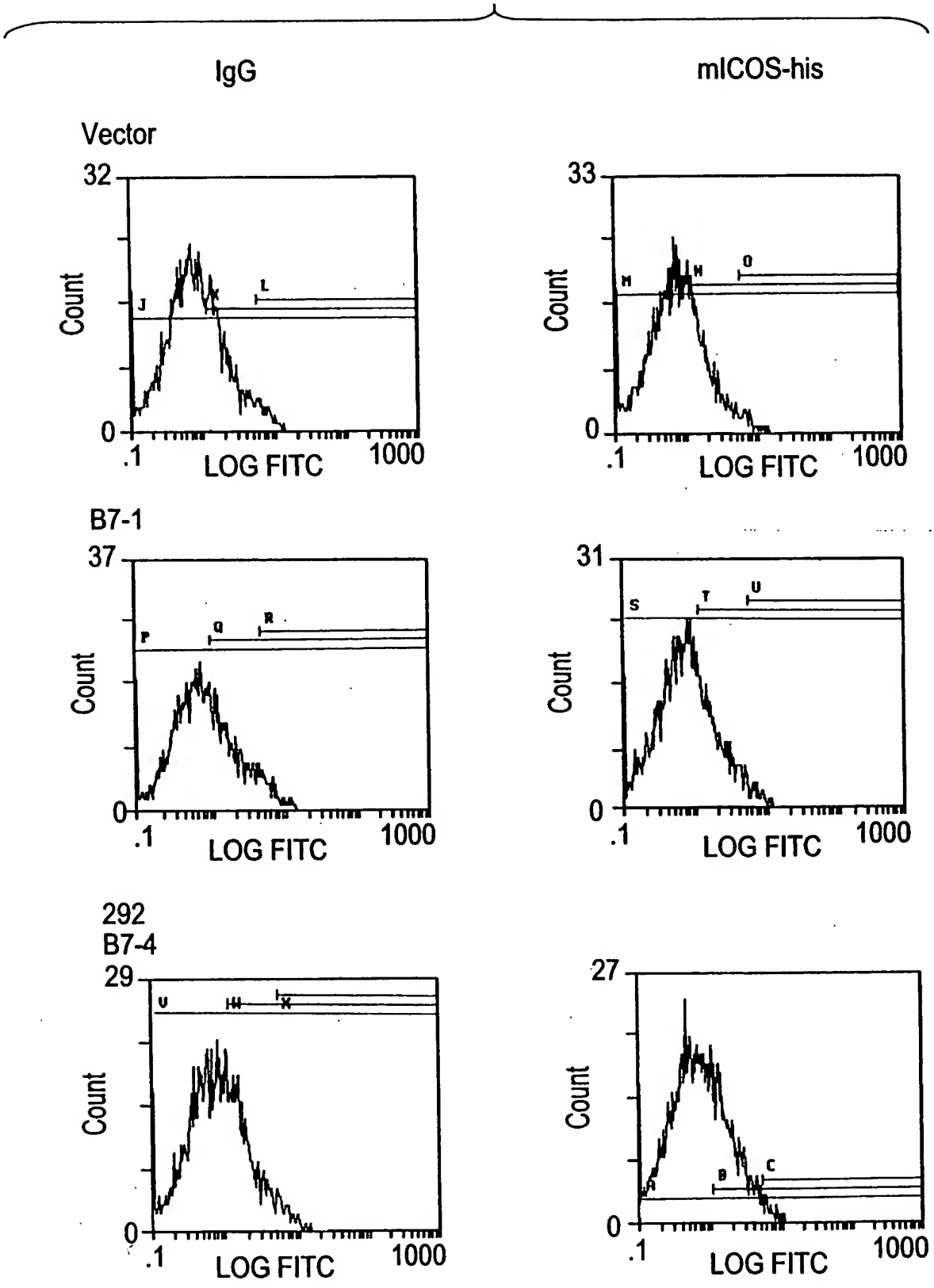

IgG
mICOS-his
Vector
32
Count
0
.1
LOG FITC
1000
J
L
33
Count
0
.1
LOG FITC
1000
M
N
O
B7-1
37
Count
0
.1
LOG FITC
1000
P
Q
R
31
Count
0
.1
LOG FITC
1000
S
T
U
292
B7-4
29
Count
0
.1
LOG FITC
1000
V
W
X
27
Count
0
.1
LOG FITC
1000
B
C

FIG. 10

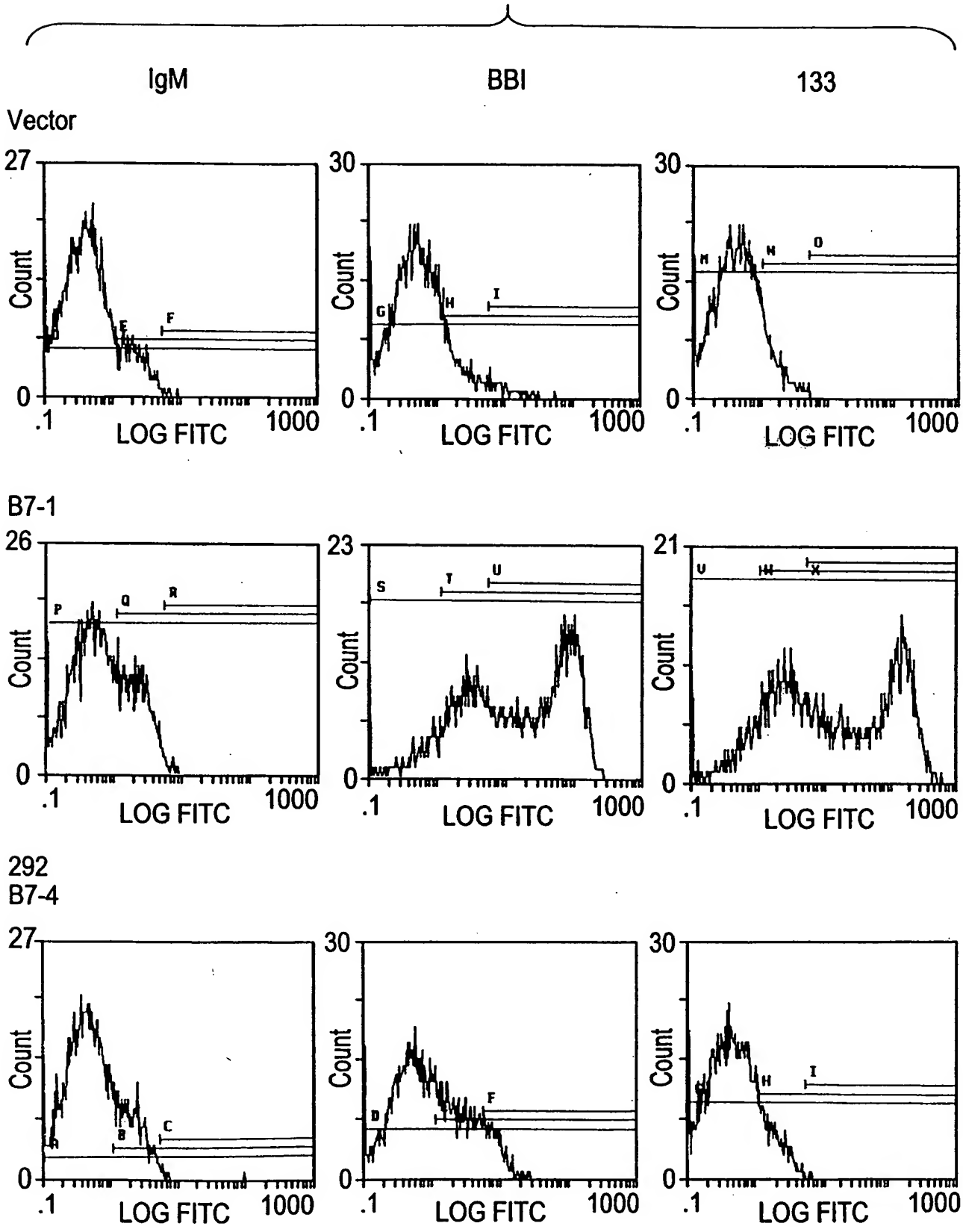

IgM
BBI
133
Vector
27
30
30
Count
LOG FITC
.1
1000
0
B7-1
26
23
21
292
B7-4
27
30
30

FIG. 11

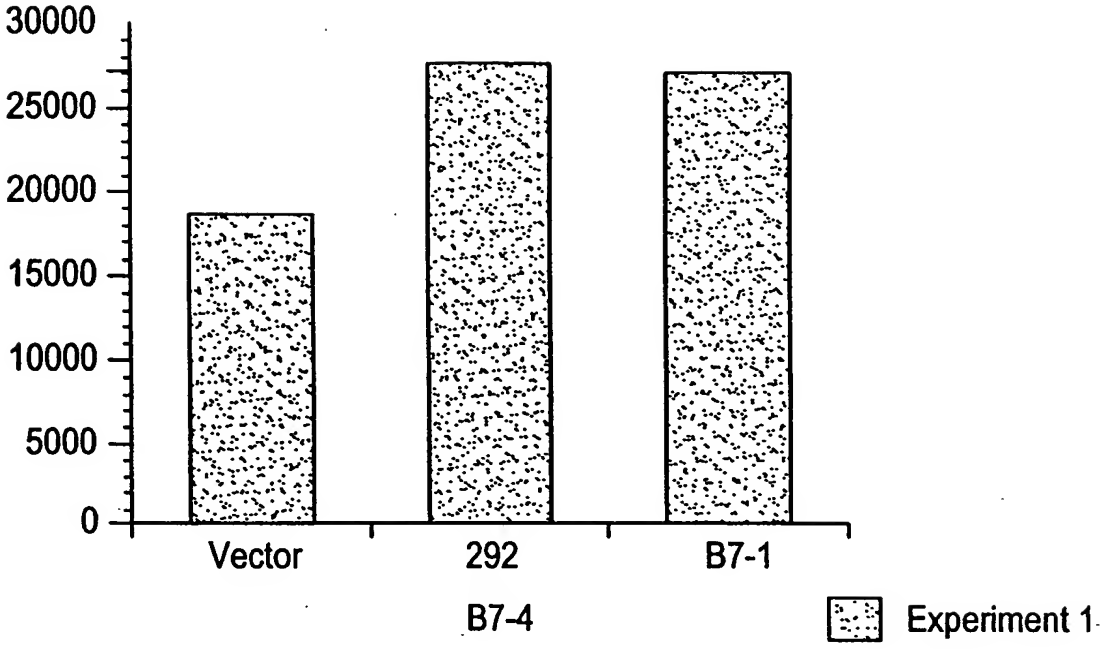

30000
25000
20000
15000
10000
5000
0
Vector
292
B7-1
B7-4
Experiment 1

FIG. 12

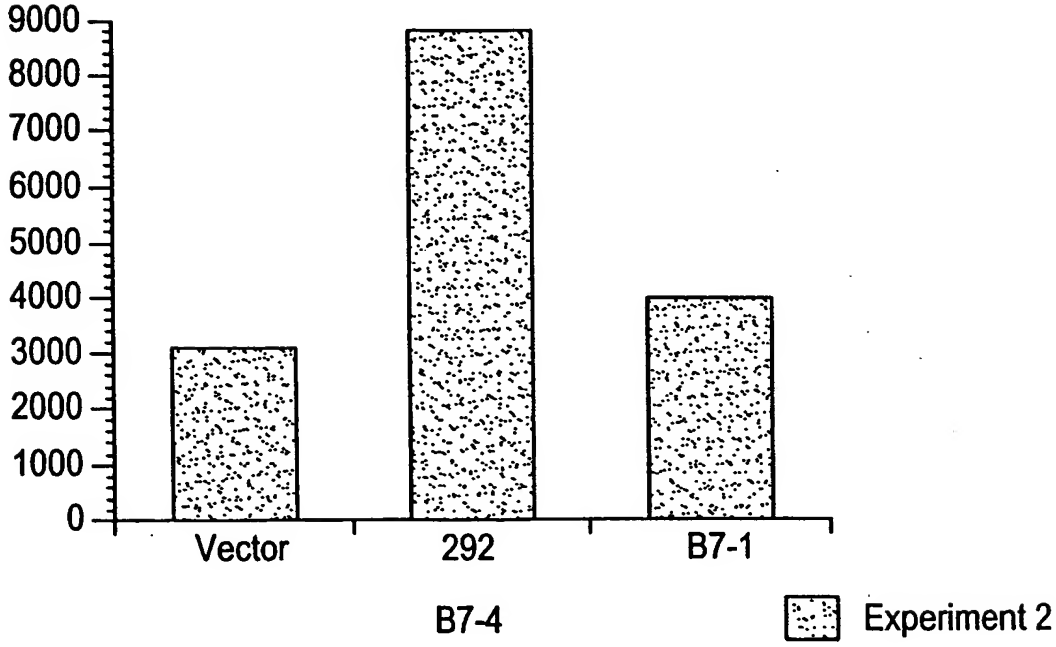

9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Vector
292
B7-1
B7-4
Experiment 2

FIG. 13A

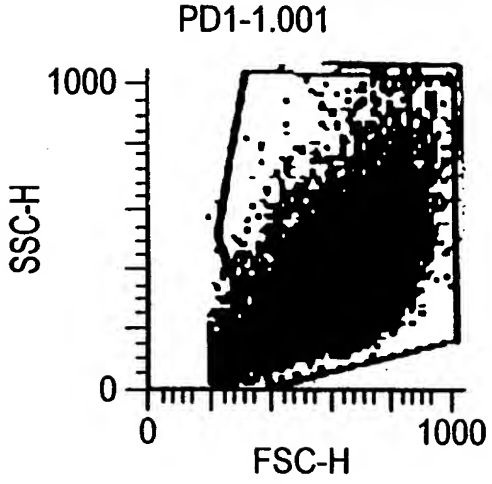


FIG. 13B

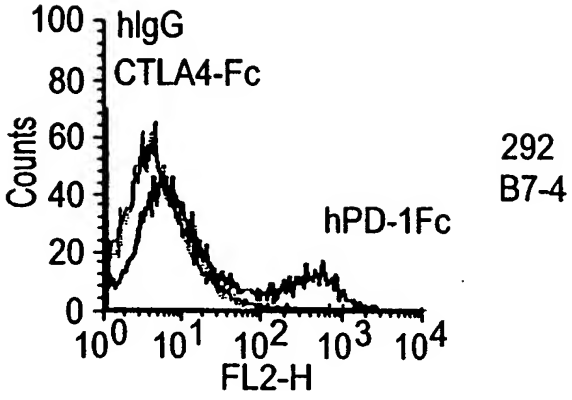


FIG. 13C

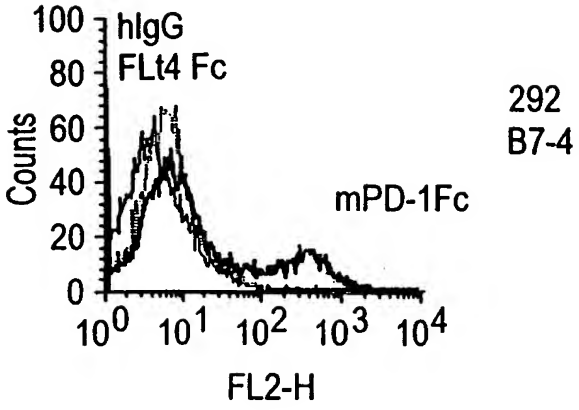


FIG. 13D

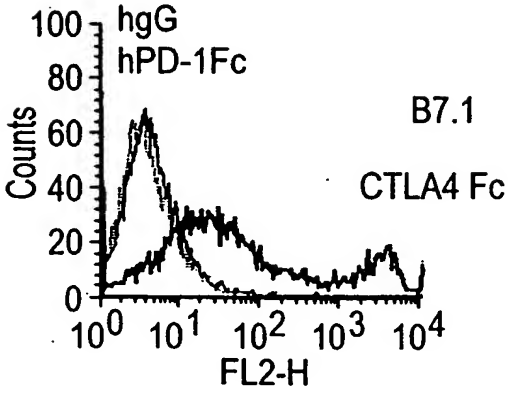


FIG. 14A

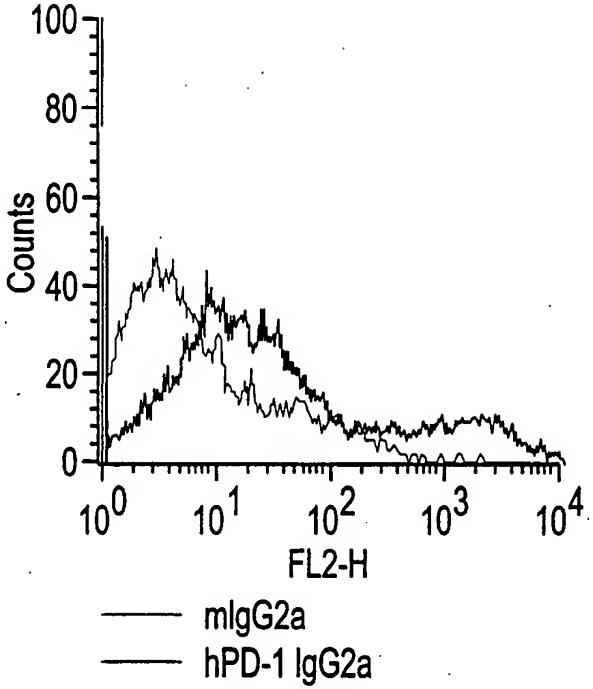


FIG. 14B

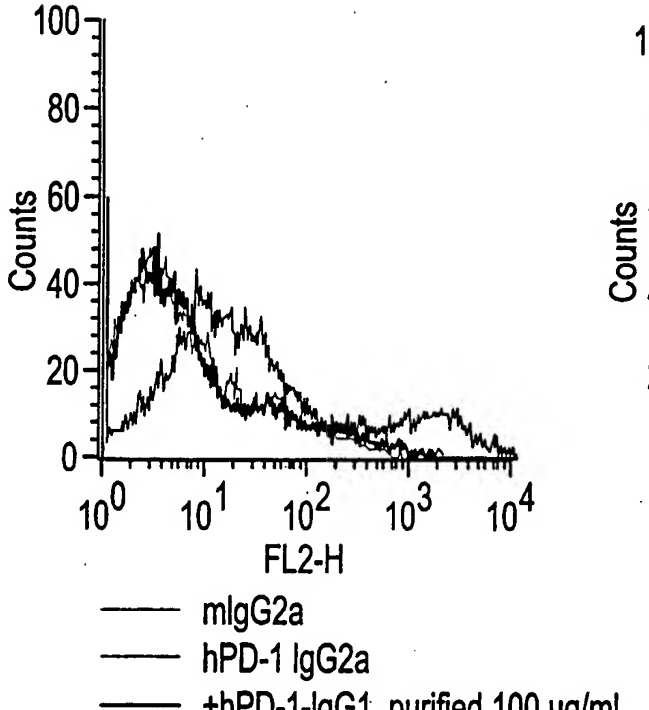


FIG. 14C

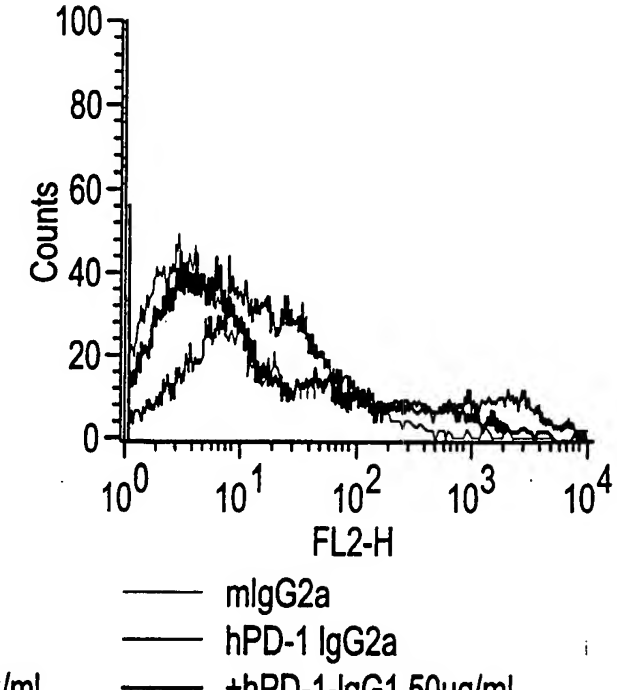


FIG. 14D

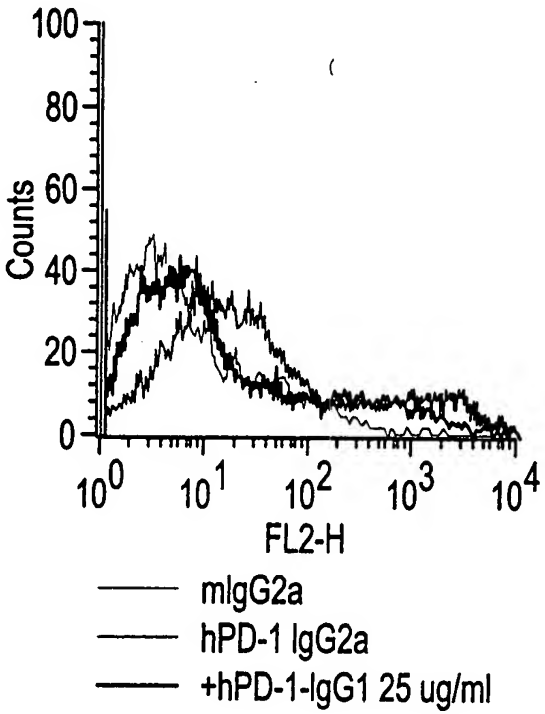


FIG. 14E

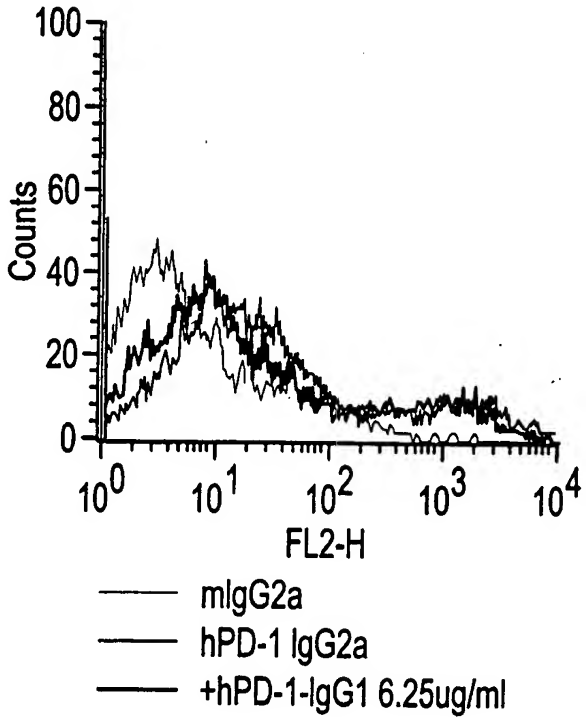


FIG. 14F

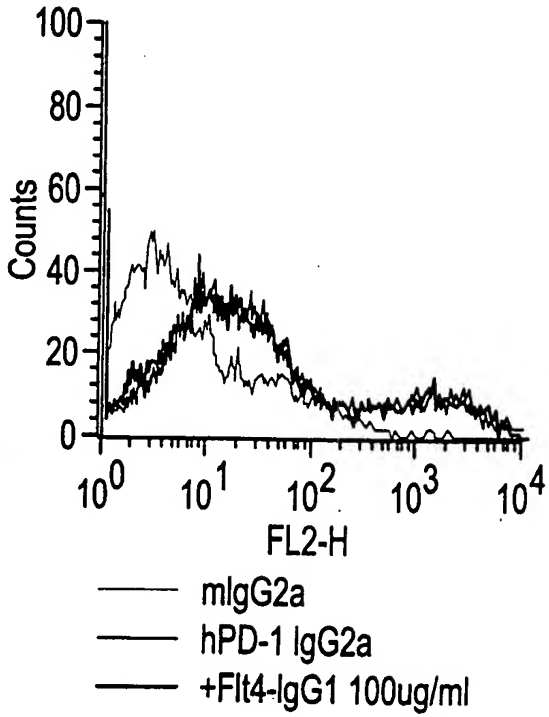


FIG. 15A

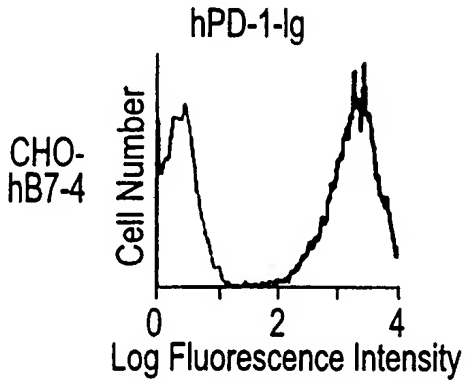


FIG. 15B

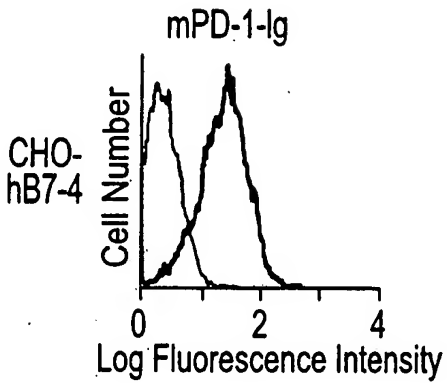


FIG. 15C

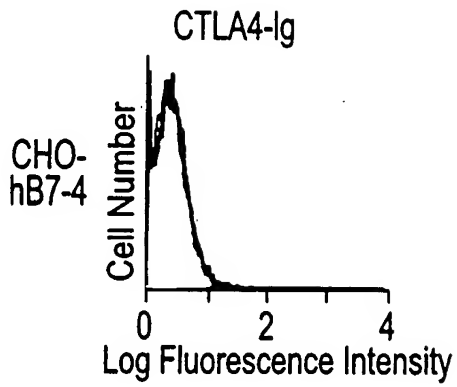


FIG. 15D

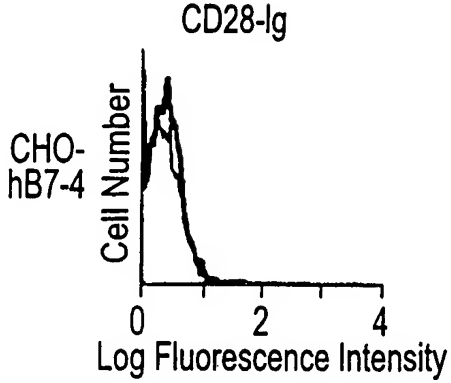


FIG. 15E

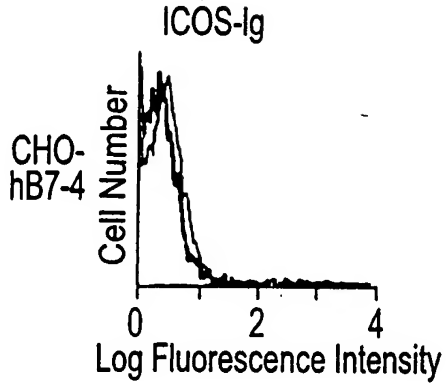


FIG. 15F

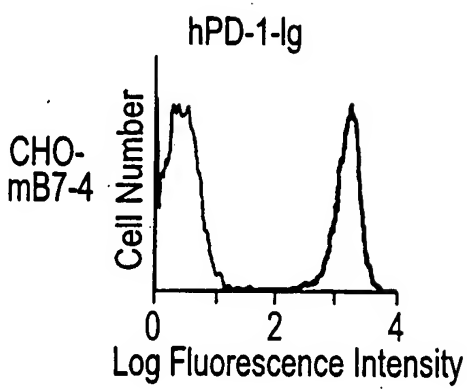


FIG. 15G

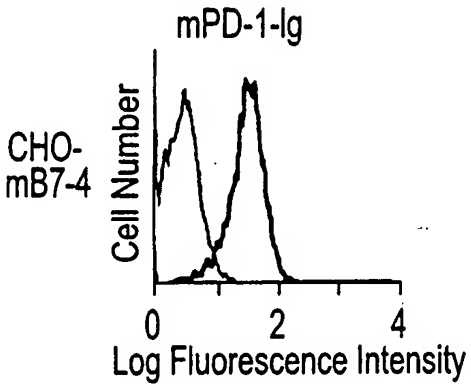


FIG. 15H

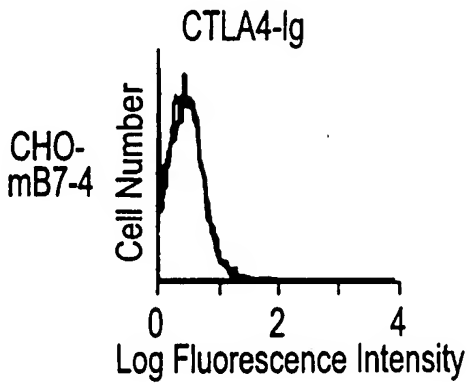


FIG. 15I

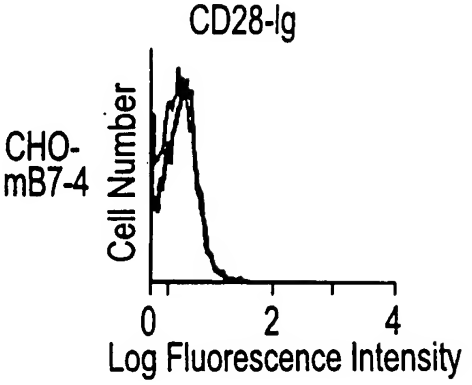


FIG. 15J

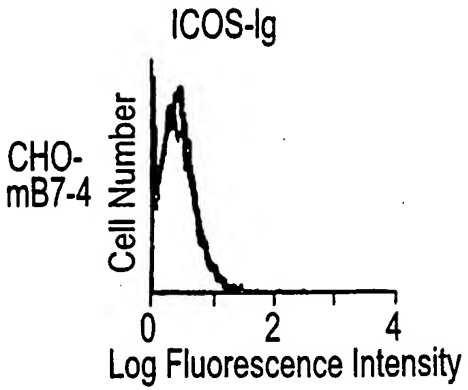


FIG. 15K

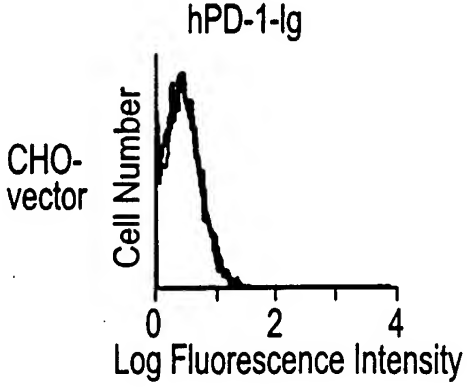


FIG. 15L

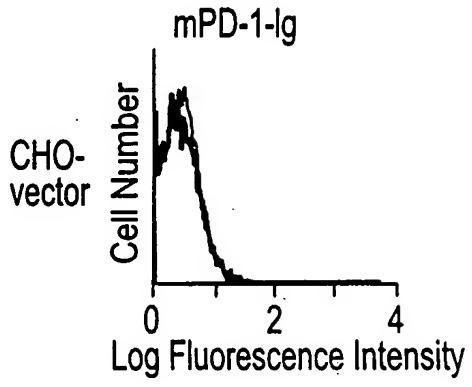


FIG. 16

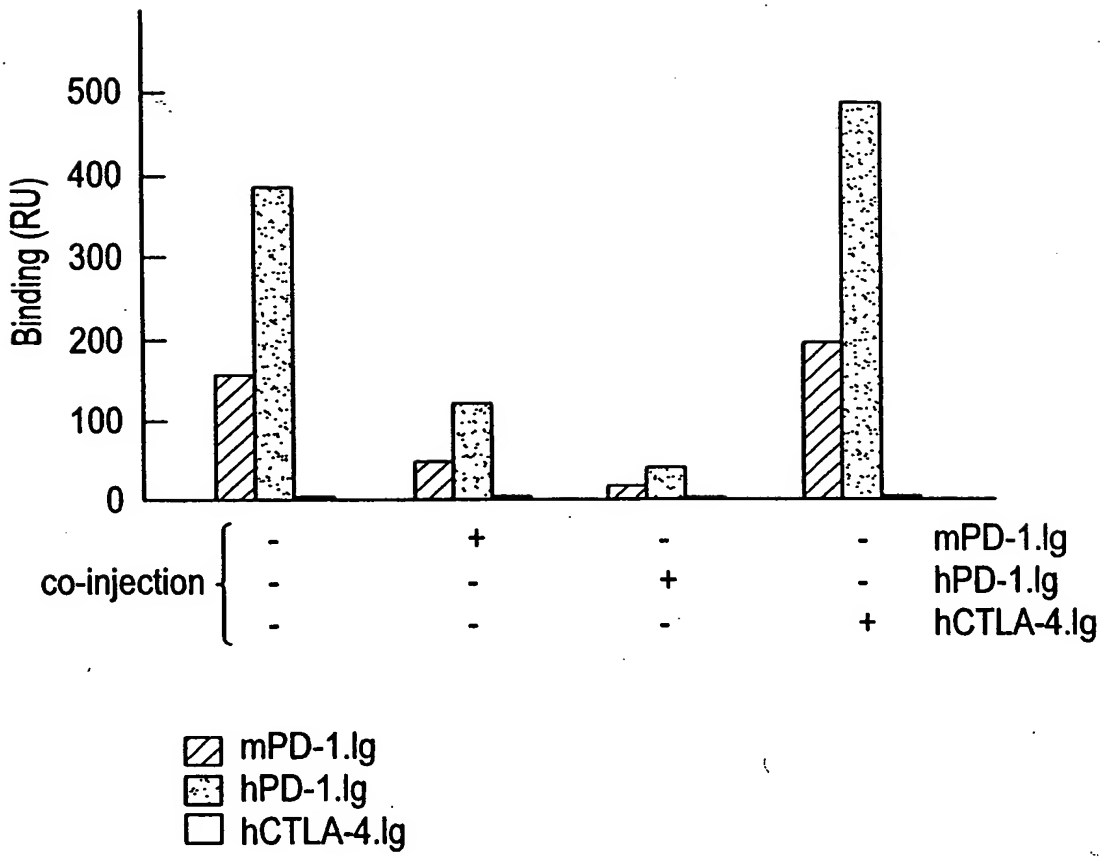

Binding (RU)
500
400
300
200
100
0
co-injection
-
+
-
-
mPD-1.lg
-
-
+
-
hPD-1.lg
-
-
-
+
hCTLA-4.lg
mPD-1.lg
hPD-1.lg
hCTLA-4.lg

FIG. 17

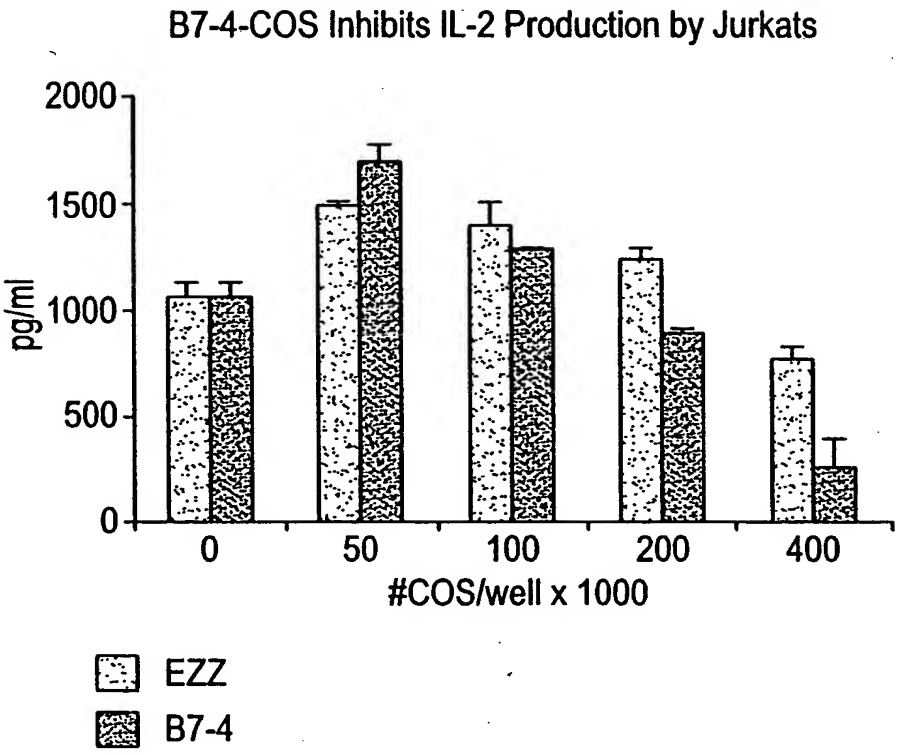

B7-4-COS Inhibits IL-2 Production by Jurkats
2000
1500
1000
500
0
pg/ml
0
50
100
200
400
#COS/well x 1000
EZZ
B7-4

FIG. 18A

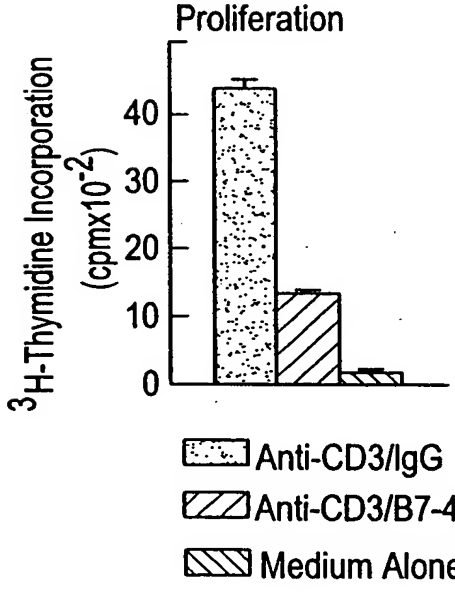


FIG. 18B

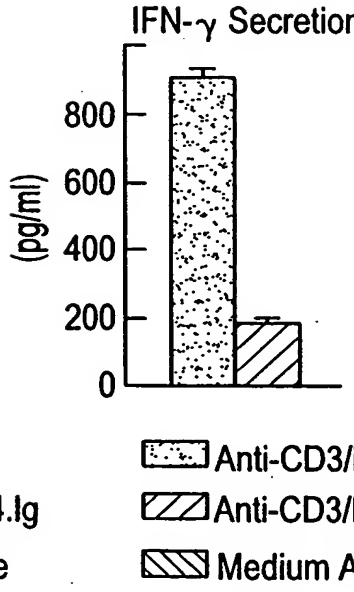


FIG. 18C

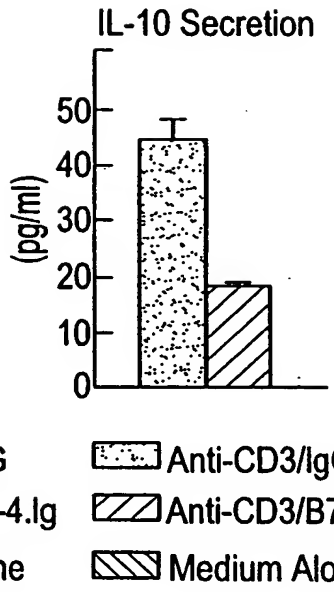


FIG. 19A

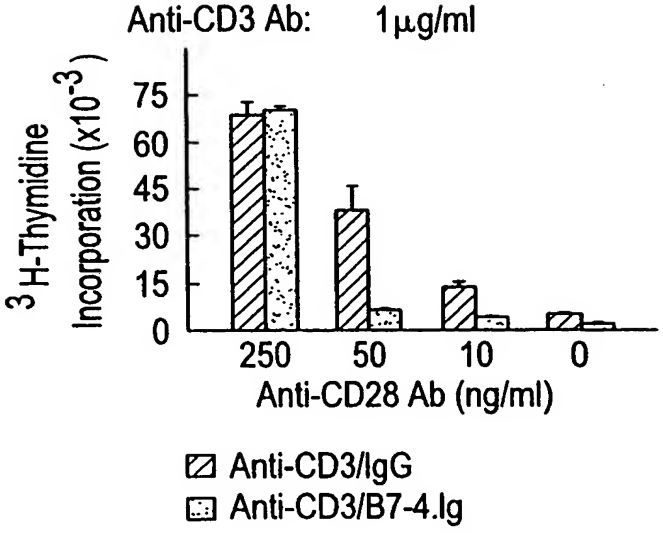


FIG. 19B

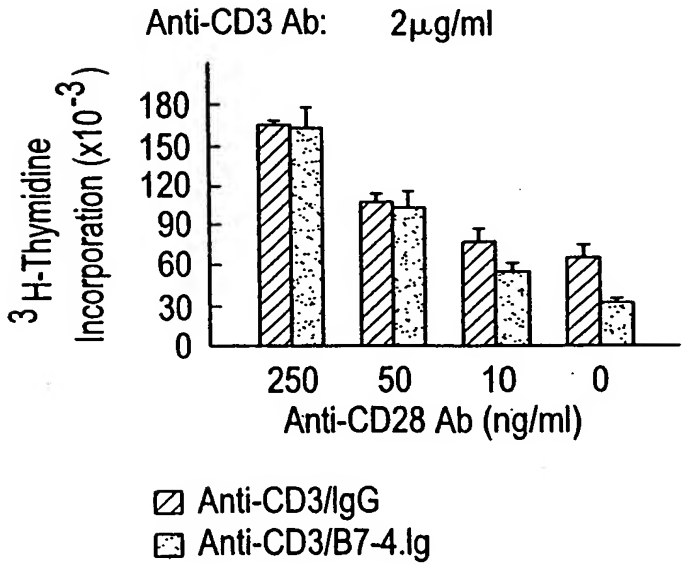


FIG. 20A

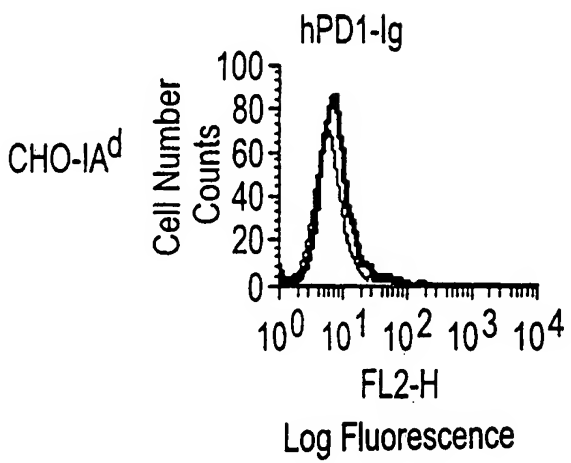


FIG. 20B

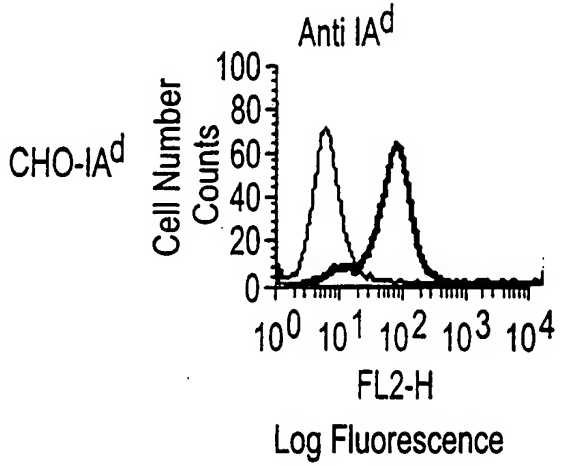


FIG. 20C

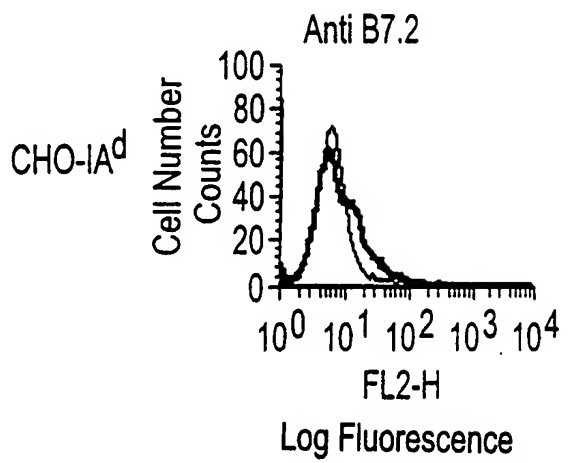


FIG. 20D

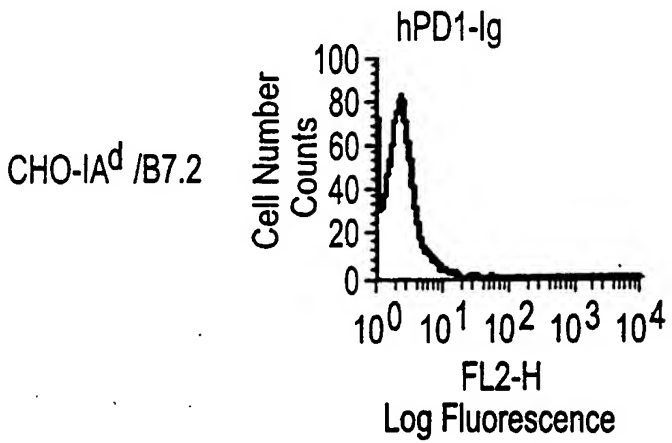


FIG. 20E

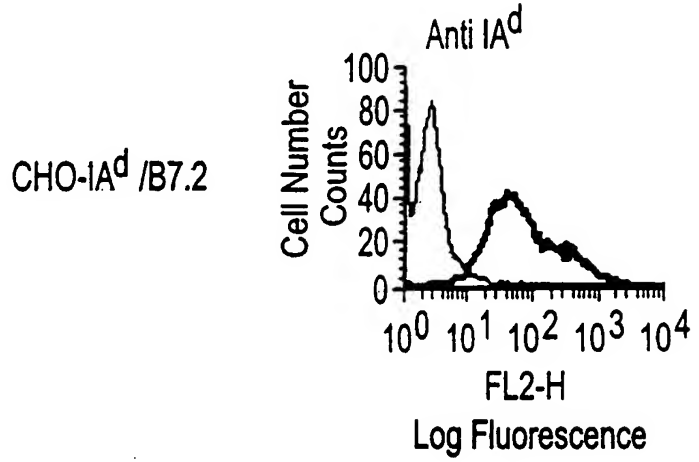


FIG. 20F

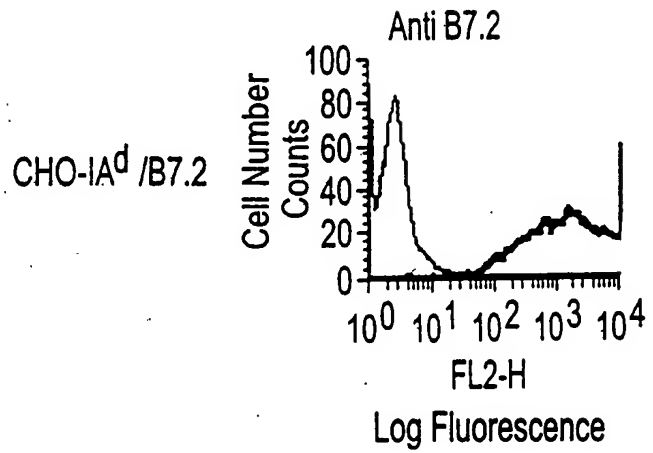


FIG. 20G

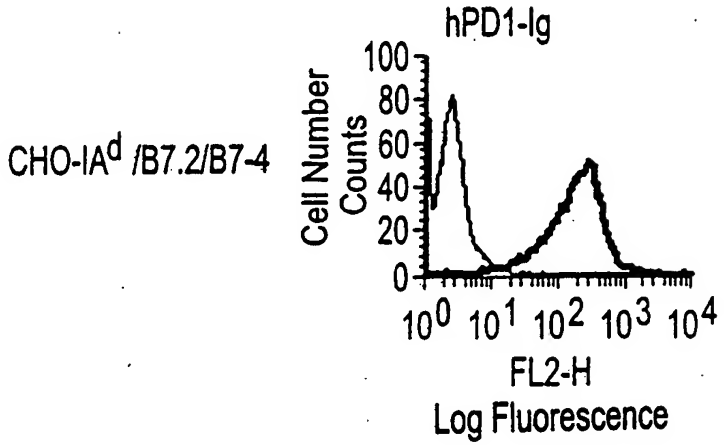


FIG. 20H

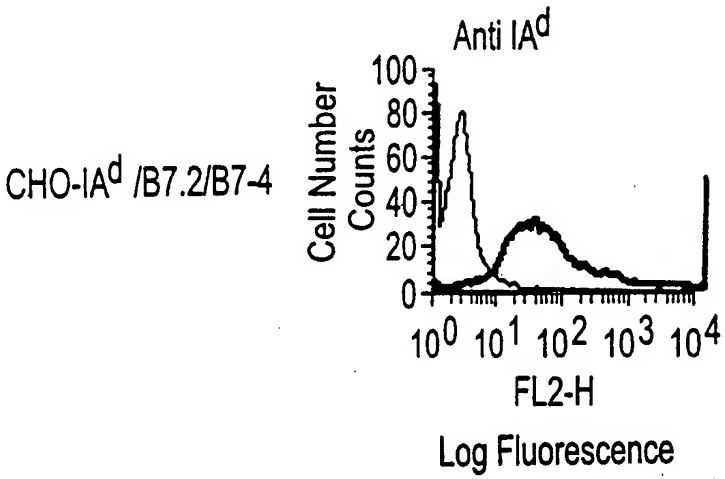


FIG. 20I

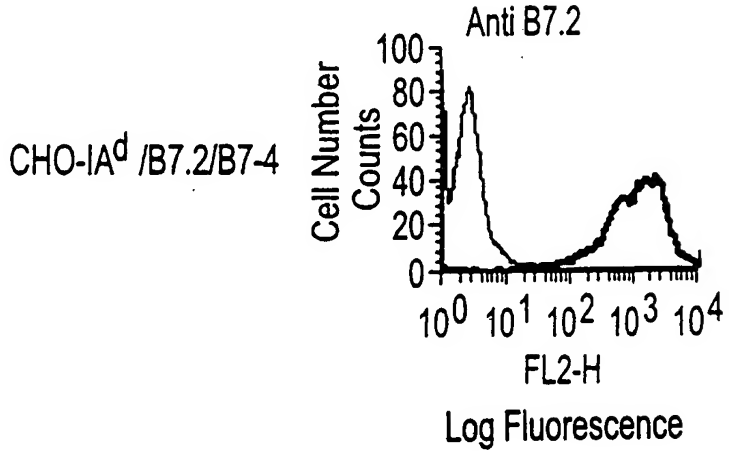


FIG. 21A

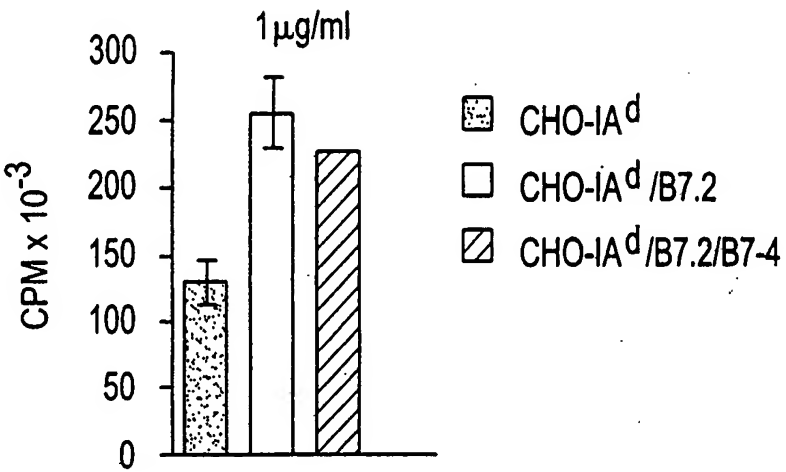


FIG. 21B

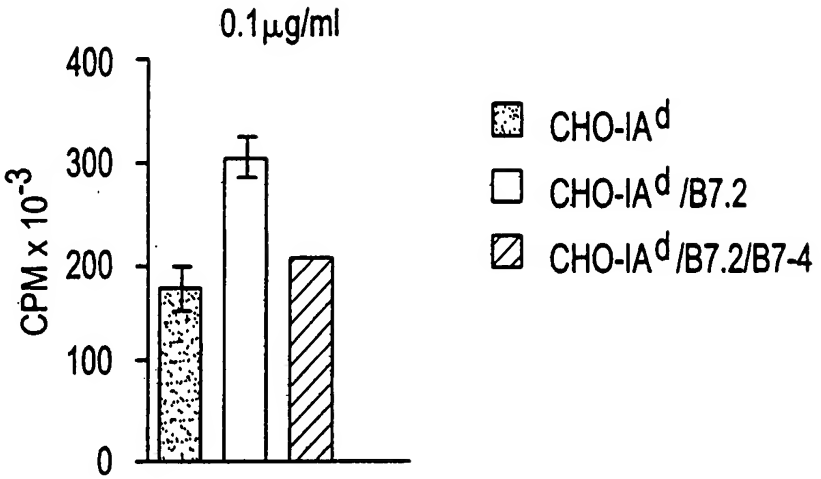


FIG. 21C

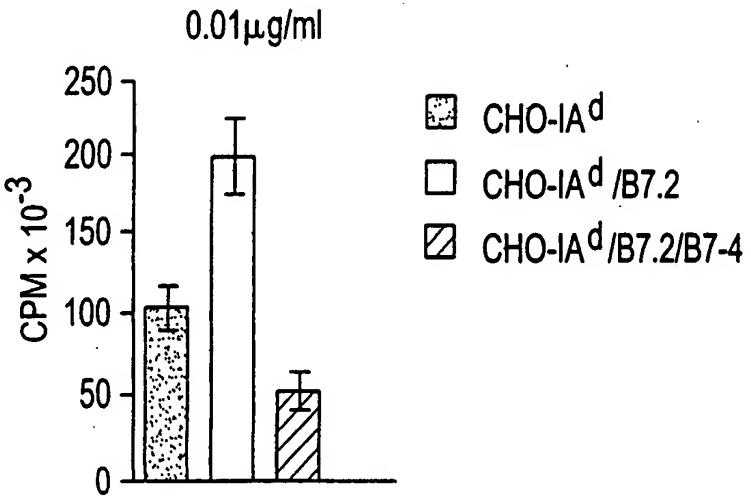


FIG.21D

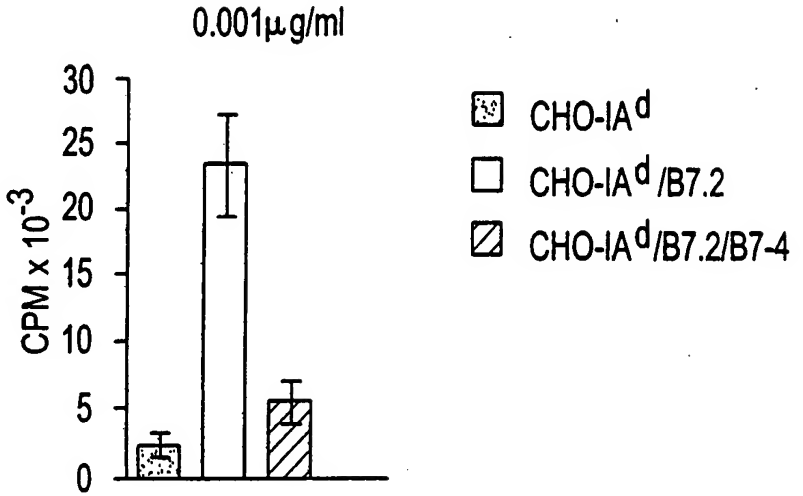


FIG. 22A

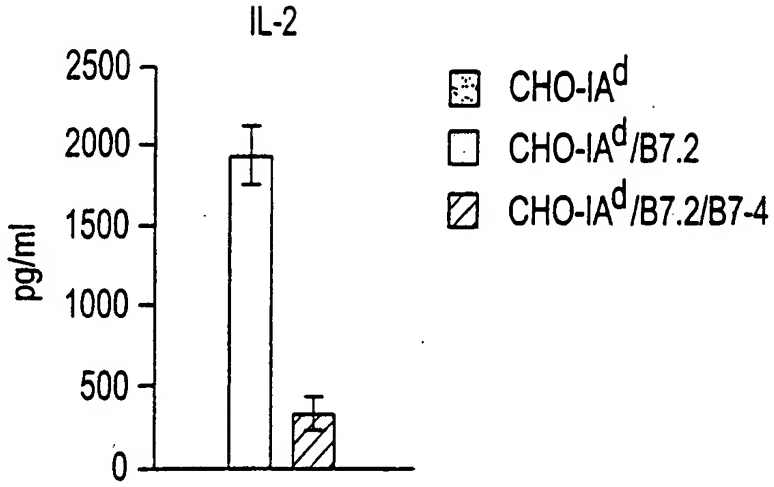


FIG. 22B

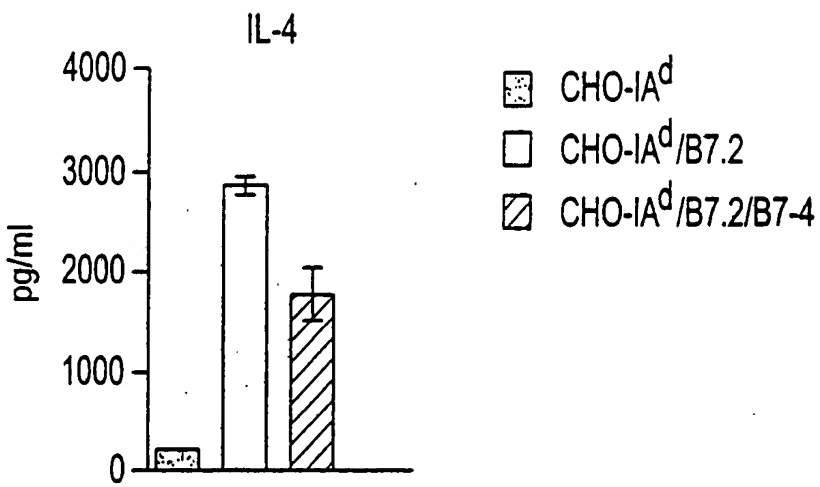


FIG. 22C

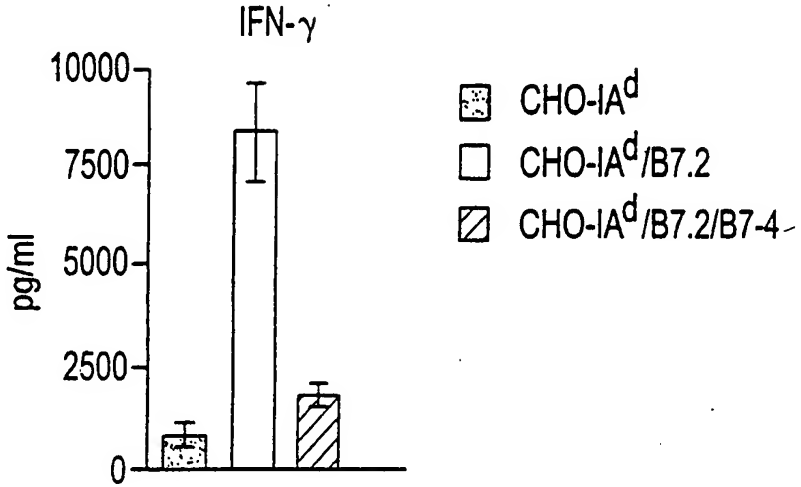


FIG. 22D

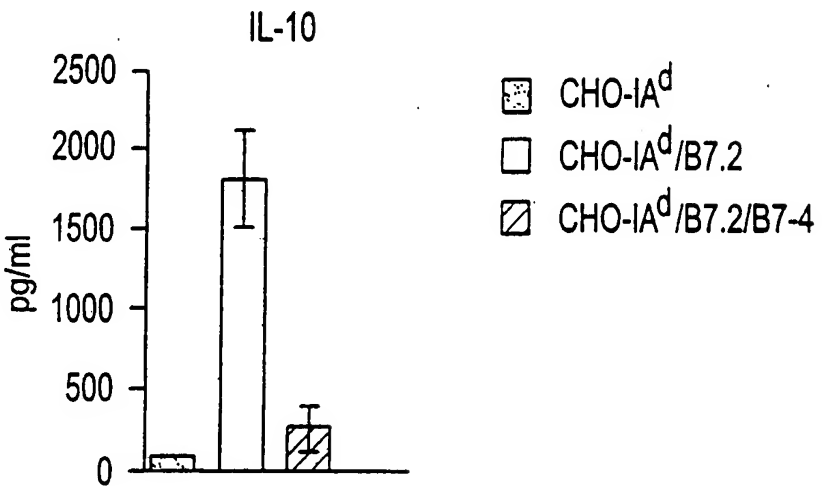


FIG. 23A

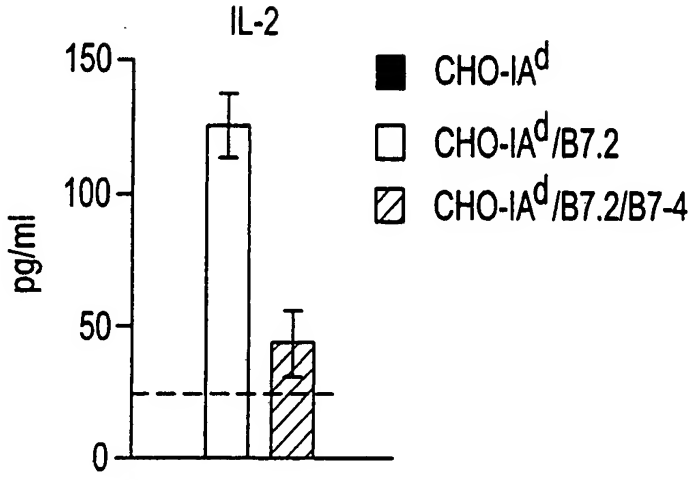


FIG. 23B

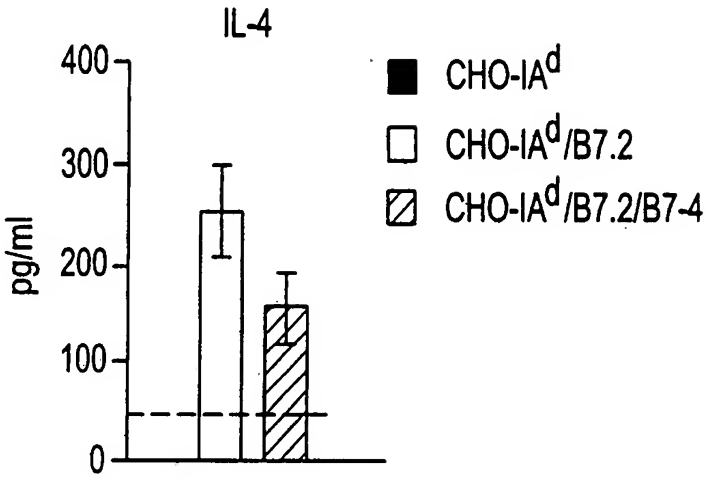


FIG. 23C

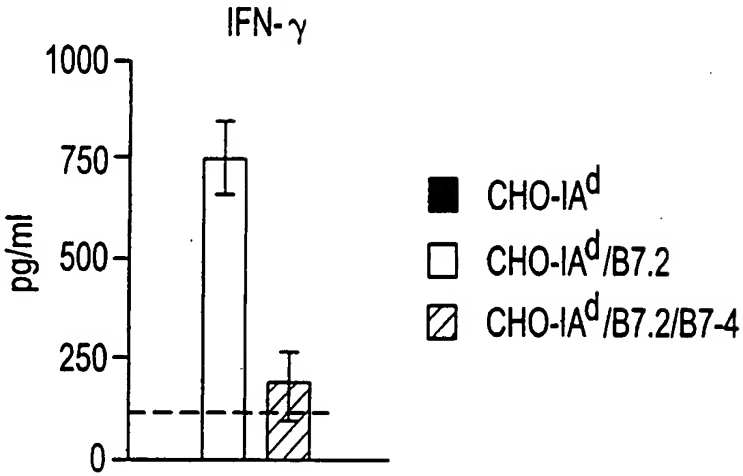


FIG. 24A

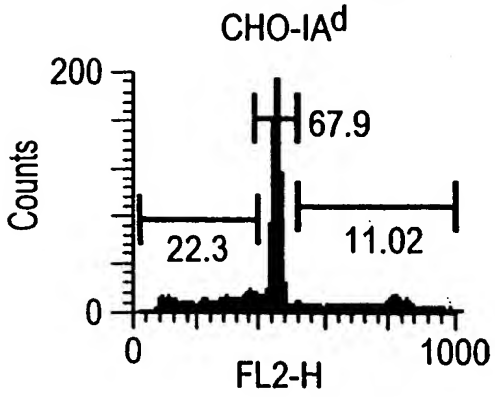


FIG. 24B

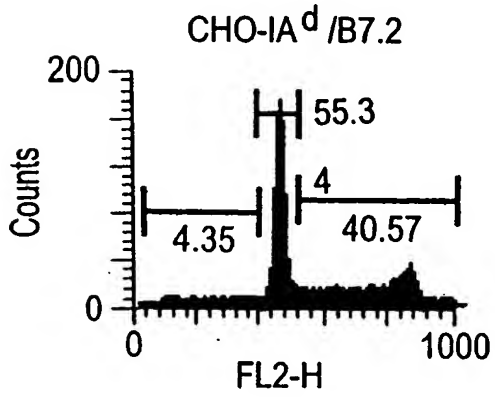


FIG. 24C

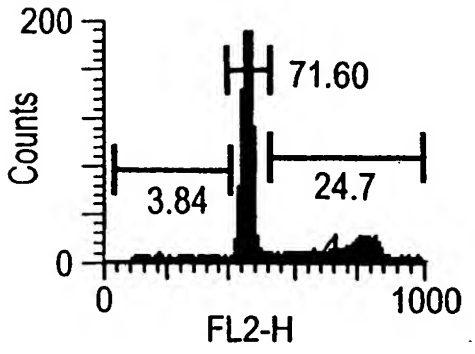


FIG. 25A

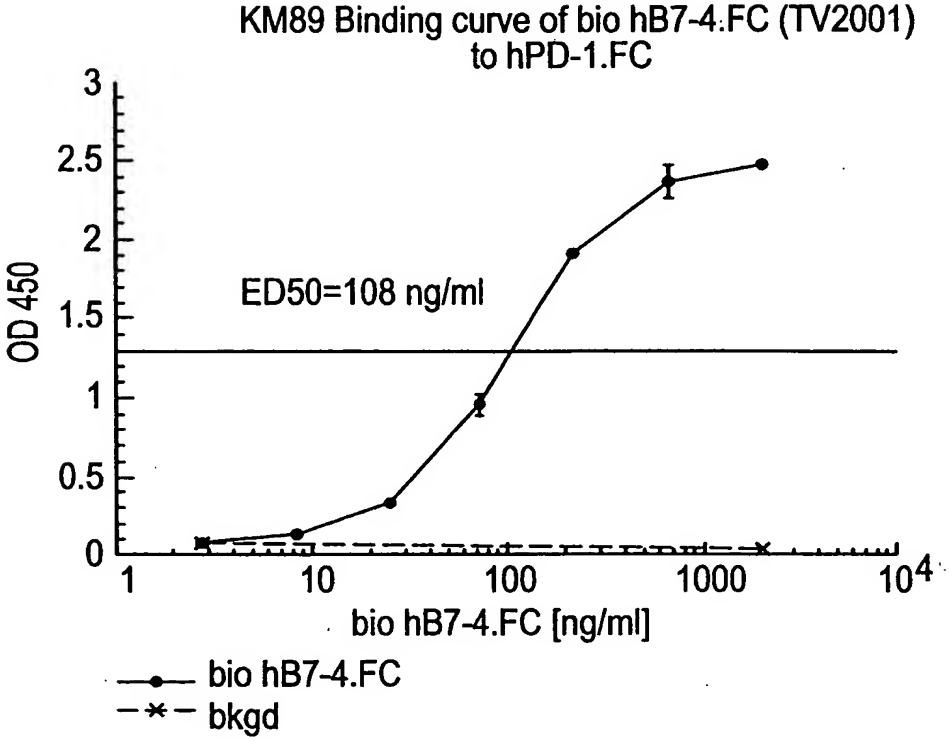
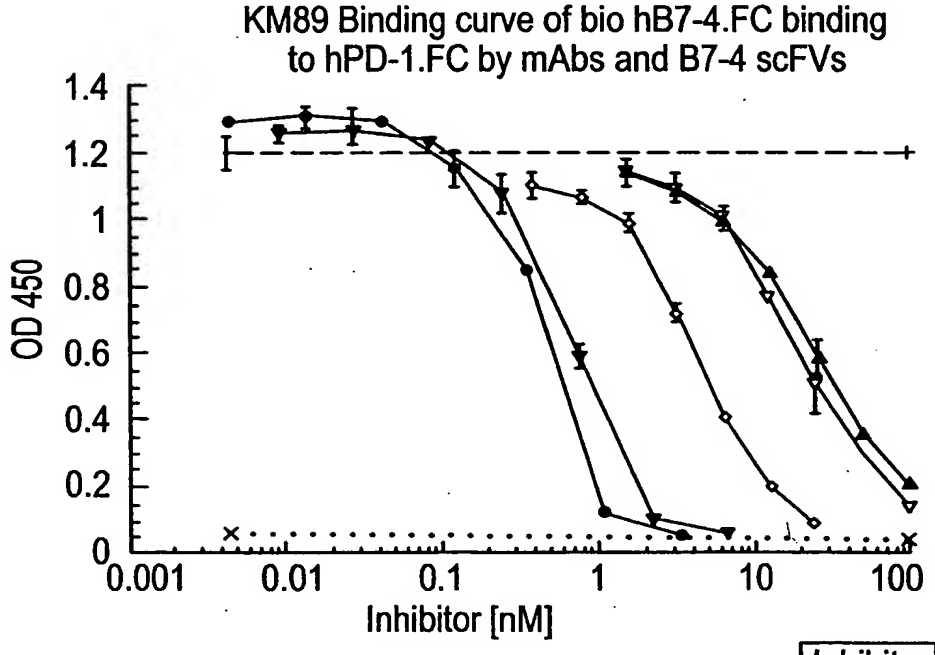


FIG. 25B



Inhibitor	IC50
10D9	0.5
11D12	0.7
B7-4-1	4
B7-4-6	19
B7-4-12	24

FIG. 26

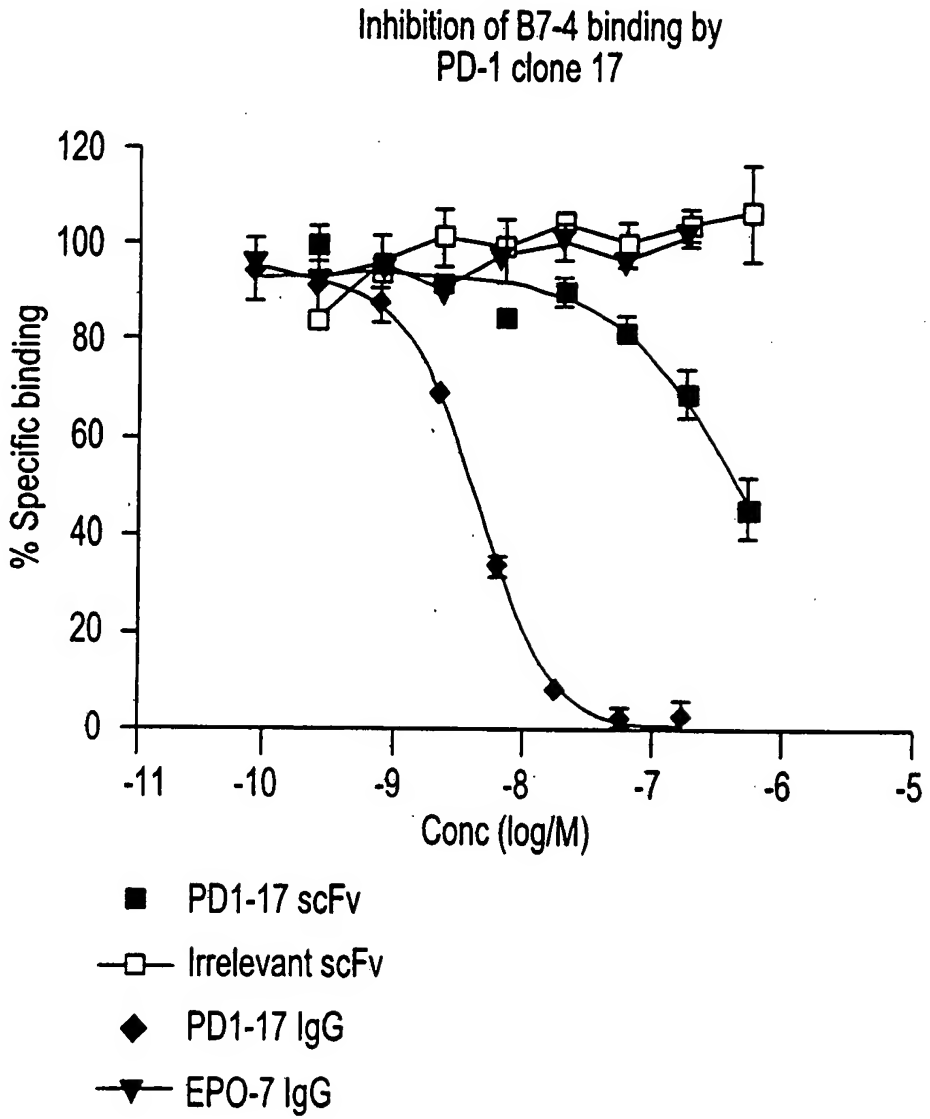

Inhibition of B7-4 binding by
PD-1 clone 17
% Specific binding
120
100
80
60
40
20
0
-11
-10
-9
-8
-7
-6
-5
Conc (log/M)
PD1-17 scFv
Irrelevant scFv
PD1-17 IgG
EPO-7 IgG

FIG. 27